

and pits were also present along the plasma membrane of the cytoplasmic processes of the stellate cells.

Scanning electron microscopy

Scanning electron microscopic (SEM) observation of the hypophysial cleft confirmed the nature of the cavity boundary cells revealed by transmission electron microscopy (TEM): a varying number of microvilli, few cytoplasmic processes and occasional cilia projecting from the cells (Fig. 7). The cilia were absent in the majority of the cells, while small concavities, possibly representing micropinocytotic vesicles, were visible here and there. In some of the cells, small bulbous protrusions with or without microvilli were occasionally found. The SEM image of the hollow in the ventral lobe was similar to that of the hypophysial cleft, though microvilli and cilia were very few.

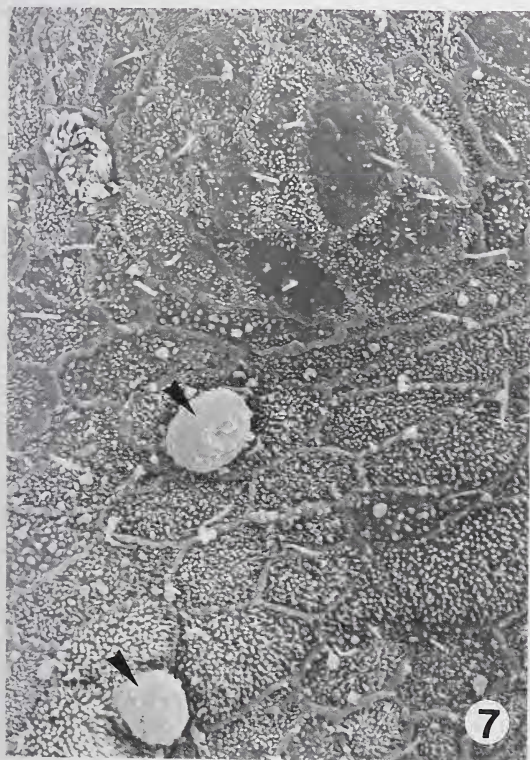


FIG. 7. Scanning electron micrograph showing apical surface of cavity boundary cells lining the hypophysial cleft. A number of microvilli, few cilia and bulbous protrusions (arrowheads) are noticeable. $\times 3,600$.

DISCUSSION

The present research on the *Mustelus* hypophysis revealed a number of the agranular cells forming a loose network in the parenchyma of the distal and ventral lobes as shown to be the case in the mammalian pars distalis [7] and the adenohypophyses of chondrosteian and holosteian fishes [5]. This organization of the cells, depicted by interconnections comprising junctional complexes and interdigitation, strongly suggests a sustentacular role for these cells.

Smaller size, lack of secretory granules and more or less paucity of cell organelles in the agranular cells suggest a possibility that some of these cells represent undifferentiated stem cells. Yoshimura *et al.* [8] have proposed a stem-cell hypothesis in the rat. According to Alluchon-Gérard [9], the embryonic hypophysis of *Scyllium* includes undifferentiated cells in addition to other agranular elements, supporting cells and pericavity cells; however, Knowles *et al.* [2] have commented nothing as to the presence of undifferentiated cells in the hypophysis of the adult *Scyliorhinus*. In the light of these results, further examination on the ontogenetic development of the gland is necessary to identify the undifferentiated cells in *Mustelus* hypophysis.

The cytological features of the agranular cells in *Mustelus*, i.e., high nucleocytoplasmic ratio, dark cytoplasm due to abundant free ribosomes, junctional complex between adjoining cells and so on, were essentially identical with those in *Scyllium* [3, 4]. Similar results were obtained in various groups of vertebrates (cyclostomes, [1, 10]; osteichthyans, [1, 5, 11, 12]; amphibians, [13, 14]; reptiles, [15, 16]; mammals, [7, 8, 17]).

In accordance with the previous findings on *Scyllium* [3, 4], two kinds of agranular cells, the cavity boundary cells and stellate cells, could be distinguished in the hypophysis of *Mustelus*. However, the "Giant cells" described in the gland of very young specimens of *Scyllium* [4] could not be found in *Mustelus*. These cells are considered to be a type of stellate cell unique to the *Scyllium* ventral lobe.

The cavity boundary cells in *Mustelus* often showed a number of periluminal vesicles with

flocculent content and contained a varying number of vacuoles and lysosome-like bodies in their cytoplasm. These aspects of the cells imply a capacity of absorption or transport of substances from the cavities into the cytoplasm and *vice versa*. In addition, it should be pointed out that microvilli, cilia, junctional complex and glycocalyx in the cavity boundary cells are common characteristics of epithelial cells, strongly reflecting their derivation from stomodeal epithelium in the embryo [5, 9].

The stellate cells of *Mustelus* showed a clear sign of phagocytotic activity. In addition, amorphous material and membranous structures in the intercellular spaces of the parenchyma may be evidence of the phagocytotic activity of the stellate cells. It is also suggested that the intercellular spaces function as a channel for metabolites to be stored in or transported into the hypophysial cavities or into the blood capillaries. To date, no similar activity of the stellate cells has been noted in elasmobranchs [2-4, 18]. However, Perryman [14] demonstrated that the stellate cells of the anuran hypophysis function as phagocytes both *in vitro* and *in vivo*. Furthermore, Båge and Fernholm [10] have suggested that the stellate cells in the proadenohypophysis of the river lamprey transform into phagocytes during late periods of gonadal maturation. Leatherland and Percy [1] and Rawdon [11] have also pointed out a phagocytotic role for the agranular cells in the teleost hypophyses under certain experimental conditions. On the other hand, Benjamin [19] has an opinion that agranular cells themselves in the hypophysis of the teleost (*Pungitius*) are a source of macrophages.

Recently, S-100 protein, a glial marker, was immunocytochemically demonstrated in the stellate cells or folliculo-stellate cells in mammalian hypophyses [20-23]. In elasmobranchs, S-100 protein-like immunoreactivity was demonstrable in the glial cells of the hypothalamo-hypophysial region and also of other brain regions, but none of the epithelial cells in the hypophysis were stained with bovine S-100 protein antiserum (Chiba *et al.*, in preparation). The most probable explanation for this negative result is that the S-100 protein-like molecule is lacking in the shark adeno-

physis.

Accordingly, the present morphological data suggest structurally and metabolically supportive functions for the agranular cells in the selachian hypophysis.

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Interpopulation Variations in Clutch Size and Egg Size in the Japanese Salamander, *Hynobius nigrescens*

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ABSTRACT—Interpopulation variations in egg size and clutch size in *Hynobius nigrescens* were studied in 32 populations from various breeding ponds in the Niigata area. As the altitude increased, the date of oviposition was delayed. However, the dates of oviposition in low elevation populations were varied independent of the altitude. The average number of eggs in an egg sac ranged from 19.5 to 70.2, and the average egg diameter ranged from 2.17 to 2.92 mm among the populations. Number and size of eggs among the populations were varied clinally. As the altitude increased, or as the date of oviposition was delayed, the average number of eggs decreased. The average egg size increased with the altitude. However, the egg size decreased with the delay in the date of oviposition among 16 lowland populations. The average total egg volume decreased with the delay in the date of oviposition. Among montane populations, however, the total egg volume showed a tendency to increase with the altitude. From the results of multiple regression analyses, the variations in the number of eggs and total egg volume were explained by the variations in the altitude and the date of oviposition. It was conceivable that the altitude and the date of oviposition were factors of the variations independent of each other. On the other hand, the variation in egg size was explained by the variations in the altitude, latitude and population size.

INTRODUCTION

Salthe [1] and Salthe and Duellman [2] showed that the interspecific variations in egg size and clutch size have been found in amphibians, and proposed that the variations in clutch size and egg size were related with the difference in the reproductive mode which might be induced by drastic environmental changes. However, the variations in egg size and clutch size have been already recognized at an intraspecific level in some amphibians [3-6]. These variations were seen in a different environment. Berven [6] compared montane and lowland populations of *Rana sylvatica*, and suggested that the variations in clutch size and egg size might be due to a different genetic basis. If the intraspecific variations in egg size and clutch size correspond with a given environment, the pattern of the variation in egg size and clutch size will change with the environment. Most previous

studies on intraspecific variations in clutch size and egg size in amphibians have been carried out on small numbers of populations. It is conceivable, however, that the variations in egg size and clutch size are caused by several complex factors. Thus, comparison among many populations may be a more effective approach in order to clarify the factors involved. Moreover, in order to understand how and why the interspecific variations in clutch size and egg size occur, we need to know the intraspecific variations in various environment.

In this paper, data on the interpopulation variations in the number and size of eggs at various altitude and dates of oviposition in a primitive salamander, *Hynobius nigrescens*, are presented and analyzed. Variations in the number of eggs with altitude are known to some extent in this species [7].

MATERIALS AND METHODS

The present study was carried out in the Niigata area in 1986 sampling 32 populations of *Hynobius*

nigrescens (Fig. 1). The breeding ponds of the populations were located at 5 to 2100 m elevation and 37° to 38° North Latitude (Table 1). Sixteen out of the 32 populations were massed at 200 m elevation and lower locations. It seems that the variations in reproductive traits among these 16 populations were not induced by the altitude. So these populations were classified into one group and named lowland populations. The other 16 populations were named montane populations.

This species breeds in lentic water, and one female lays a pair of egg sacs. Thus, the mean number of eggs in an egg sac was used to substitute for the clutch size in the present paper. The mean number of eggs in an egg sac in each population was calculated from the number of eggs in 20 egg sacs.

Embryos at the blastula or gastrula stage, spherical and egg-like in appearance, were measured under a stereo-microscope to the nearest 0.01 mm. These embryos gradually increase in size with the advance of development, so the initial (uncleaved) egg diameter was calculated from the embryo size using the conversion factors established for this

species [8]. Data on the mean egg size in each population were taken from the mean egg diameter in 10 egg sacs. The mean egg diameter in each egg sac was based on 10 specimens taken from the central part of the egg sac. Data on the mean total egg volume in each population are based on the total egg volume (egg volume \times number of eggs in an egg sac \times 2) in 10 egg sacs. The egg volume was calculated from the diameter using the equation for sphere volume.

Population size was estimated by counting the number of egg sacs, at the end of the breeding season. The dates of oviposition were estimated from the embryonic stages and the degree of swelling of the egg sacs, while the mean date of oviposition in each population was calculated from the dates of oviposition of the egg sacs that were used for the measurement of egg size. For statistical analyses, the dates of oviposition must be quantitated, so the date of oviposition in the first breeding population was expressed as 1, and dates in other populations were expressed as 1 plus the number of days after 1.

Statistical analyses were performed using NEC statistics program package (STATPAC). The significance of egg size difference among whole populations, among lowland and among montane populations were analyzed by the Kruskal-Wallis test. Spearman rank correlations were employed to test relationships between two variables. The multiple regression technique was used to explain the variations in egg number, egg size and total egg volume.

RESULTS

1. Breeding period

The relationship between the breeding period and altitude for each population is illustrated in Figure 2. The breeding period in each population closely corresponded with the thawing of the snow. Populations examined in the present study included various types, from one of the earliest breeding populations to one of the latest breeding ones in Niigata area. The date of oviposition varied up to 4 months among all the populations examined, and up to 2 months among just the

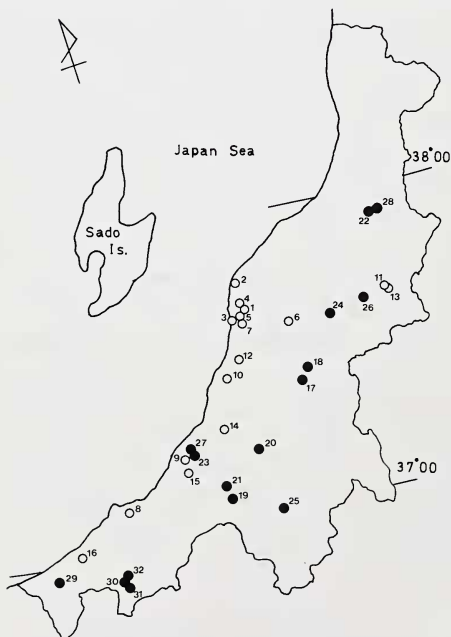


FIG. 1. Map of Niigata Prefecture showing sample sites. Open circle: lowland population, Solid circle: montane population.

TABLE 1. Characters in each population

Population number	Altitude (m)	Latitude	Mean date of oviposition	Number of egg sacs	Condition of breeding pond ¹⁾
Lowland	population				
1	20	37°44'	Mar. 21	48	Shallow pool with constant inflow
2	5	37°48'	Mar. 25	164	Artificial pond near the coast
3	170	37°42'	Mar. 28	72	Farm pond
4	180	37°44'	Apr. 3	ca.1000	Artificial pond
5	60	37°43'	Apr. 3	190	Artificial pond with constant inflow
6	100	37°37'	Apr. 8	130	Large pond with constant inflow
7	40	37°41'	Apr. 14	ca.200	Small artificial pond
8	60	37°08'	Apr. 21	54	Farm pond
9	140	37°16'	Apr. 24	20	Farm pond
10	100	37°30'	Apr. 24	202	Shallow farm pond
11	150	37°40'	Apr. 25	20	Abandoned rice field
12	140	37°32'	Apr. 29	ca.1500	Large farm pond
13	150	37°40'	May 1	110	Artificial pond
14	140	37°21'	May 3	71	Abandoned rice field
15	80	37°13'	May 3	84	Farm pond
16	130	37°01'	May 9	246	Large pond owed to spring water
Montane	population				
17	300	37°25'	May 4	52	Small farm pond
18	270	37°25'	May 4	90	Abandoned rice field
19	330	37°05'	May 12	32	Farm pond
20	250	37°15'	May 12	136	Farm pond
21	250	37°09'	May 22	200	Farm pond
22	740	37°54'	May 25	30	Small pond
23	520	37°16'	May 29	594	Middle-sized pond
24	1012	37°37'	June 1	1050	Small pond
25	830	37°01'	June 10	190	Middle-sized pond
26	650	37°39'	June 12	ca.1800	Middle-sized pond
27	630	37°17'	June 13	482	Middle-sized pond
28	1060	37°54'	June 16	ca.800	Middle-sized pond
29	680	36°58'	June 25	1112	Middle-sized pond
30	2080	36°53'	July 9	660	Small ponds in marshy plain
31	2010	36°54'	July 9	714	Small ponds in marshy plain
32	2100	36°53'	July 9	208	Large pond in marshy plain

¹⁾ Small pond: under 25 m², Middle-sized pond: 25–200m², Large pond: over 200 m²

lowland populations. In general, the duration of breeding was longer in lowland (the longest was about 2 months) and shorter in montane populations (the longest was 2 weeks). So far as lowland populations are concerned, there was no apparent relationship between the date of oviposition and altitude ($n=16$, $r_s=0.324$). In these cases, the

date of oviposition was determined by the time of thawing. However, among montane populations ($n=16$, $r_s=0.847$, $P<.01$), and among all the populations examined ($n=32$, $r_s=0.889$, $P<.01$), a significant correlation between the date of oviposition and altitude was recognized statistically.

Among the sampling sites in the present study,